

## **AMENDMENT TO THE CLAIMS**

Please amend the claims as shown below. This listing of claims will replace all prior versions and listings of claims in the application. Deletions of text are indicated with ~~strikethrough~~ or double bracket [[xxx]]. Added text is shown by underline. Claim status is indicated as **withdrawn**, **currently amended**, **original**, **previously presented** or **cancelled**.

### **LISTING OF THE CLAIMS:**

1. **(withdrawn)** A composition comprising an orthogonal leucyl-tRNA (leucyl-O-tRNA), wherein the leucyl O-tRNA comprises an anticodon loop comprising a CU(X)<sub>n</sub> XXXAA sequence, and comprises at least about a 25% suppression activity in presence of a cognate synthetase in response to a selector codon as compared to a control lacking the selector codon.
2. **(withdrawn)** The composition of claim 1, wherein the leucyl-O-tRNA comprises a stem region comprising matched base pairs and a conserved discriminator base at position 73 and wherein the selector codon is amber codon.
3. **(withdrawn)** The composition of claim 2, wherein the CU(X)<sub>n</sub> XXXAA sequence comprises CUCUAAA sequence and n=0.
4. **(withdrawn)** The composition of claim 2, wherein the leucyl-O-tRNA comprises a C:G base pair at position 3:70.
5. **(withdrawn)** The composition of claim 1, wherein the leucyl-O-tRNA comprises: a first pair selected from the group consisting of: U28:A42, G28:C42 and C28:G42; and, a second pair selected from the group consisting of: G:49:C65 or C49:G65; and, wherein the selector codon is a four-base codon.
6. **(withdrawn)** The composition of claim 5, wherein the CU(X)<sub>n</sub> XXXAA sequence comprises a CUUCCUAA sequence and n=1.
7. **(withdrawn)** The composition of claim 5, wherein the first pair is C28:G42 and the second pair is C49:G65.
8. **(withdrawn)** The composition of claim 1, wherein the CU(X)<sub>n</sub> XXXAA sequence comprises a CUUCAAA sequence and n=0, and wherein the selector codon is an opal codon.

9. **(withdrawn)** The composition of claim 1, wherein the leucyl-O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in any one of SEQ ID NO.: 3, 6, 7 or 12, or a complementary polynucleotide sequence thereof.

10. **(withdrawn)** The composition of claim 1, wherein the leucyl-O-tRNA and cognate synthetase, or a conservative variant thereof, are at least 50% as effective at suppressing a selector codon as a leucyl O-tRNA of SEQ ID NO: 3, 6, 7 or 12, in combination with a cognate synthetase.

11. **(withdrawn)** The composition of claim 1, further comprising an orthogonal leucyl aminoacyl-tRNA synthetase (leucyl O-RS), wherein the leucyl O-RS preferentially aminoacylates the leucyl-O-tRNA with a selected amino acid.

12. **(withdrawn)** The composition of claim 11, wherein the leucyl O-RS, or a portion thereof, is encoded by a polynucleotide sequence as set forth in any one of SEQ ID NO.: 13 or 14, or a complementary polynucleotide sequence thereof.

13. **(withdrawn)** The composition of claim 11, wherein the leucyl O-RS comprises an amino acid sequence as set forth in any one of SEQ ID NO.: 15 or 16, or a conservative variation thereof.

14. **(withdrawn)** The composition of claim 1, wherein the leucyl-O-tRNA is derived from an archaeal tRNA.

15. **(withdrawn)** The composition of claim 1, wherein the leucyl-O-tRNA is derived from *Halobacterium sp NRC-1*.

16. **(withdrawn)** The composition of claim 1, further comprising a translation system.

17. **(currently amended)** A cell comprising a translation system, wherein the translation system comprises:

an orthogonal leucyl-tRNA (leucyl-O-tRNA), ~~wherein the leucyl-O-tRNA comprises comprising a nucleotide sequence selected from SEQ ID NOS: 1, 2, 4-7 and 12, at least about a 25% suppression activity in presence of a cognate synthetase in response to a selector codon as compared to a control lacking the selector codon;~~ an orthogonal aminoacyl-leucyl-tRNA synthetase (leucyl-O-RS); and, a first selected amino acid;

wherein the leucyl O-tRNA comprises an anticodon loop comprising a CU(X)nXXXXAA sequence and recognizes a [[the]] first selector codon, and the leucyl O-RS preferentially aminoacylates the leucyl O-tRNA with the first selected amino acid.

**18. (currently amended)** The cell of claim 17, wherein the leucyl O-tRNA comprises or is encoded by a polynucleotide sequence as set forth in any one of SEQ ID NO.: 3, 6, 7 or 12, or a complementary polynucleotide sequence thereof, and wherein the leucyl O-RS comprises an amino acid sequence as set forth in any one of SEQ ID NO.: 15, [[or]] 16, and or a conservative variation thereof.

**19. (currently amended)** The cell of claim 17, wherein the leucyl-O-tRNA and leucyl-O-RS cognate synthetase, or a conservative variant thereof, are at least 50% as effective at suppressing the first [[a]] selector codon as a leucyl O-tRNA of SEQ ID NO: 1, 2, 4-7 or 12 3, 6, 7 or 12, in combination with a leucyl O-RS comprising an amino acid sequence as set forth in SEQ ID NO.: 15 or 16 cognate synthetase.

**20. (currently amended)** The cell of claim 17, wherein the cell further comprises an additional different O-tRNA/O-RS pair and a second selected amino acid, wherein the additional O-tRNA recognizes a second selector codon and the additional O-RS preferentially aminoacylates the additional O-tRNA with the second selected amino acid.

**21. (currently amended)** The cell of claim 17, wherein the leucyl O-tRNA is derived from *Halobacterium sp NRC-1* and the leucyl O-RS is derived from *Methanobacterium thermoautotrophicum thermoautotrophicum*.

**22. (original)** The cell of claim 17, wherein the cell is a eukaryotic cell.

**23. (original)** The cell of claim 17, wherein the cell is a non-eukaryotic cell.

**24. (original)** The cell of claim 23, wherein the non-eukaryotic cell is an *E. coli* cell.

**25. (currently amended)** The cell of claim 17, further comprising a nucleic acid that comprises a polynucleotide that encodes a polypeptide of interest, wherein the polynucleotide comprises or encodes at least one codon that is the first [[a]] selector codon that is recognized by the leucyl O-tRNA.

**26. (currently amended)** An *E. coli* cell comprising:

an orthogonal leucyl-tRNA (leucyl-O-tRNA), wherein the leucyl-O-tRNA comprises a nucleotide sequence selected from SEQ ID NOS: 1, 2, 4-7 and 12, at least about a

~~25% suppression activity in presence of a cognate synthetase in response to a selector codon as compared to a control lacking the selector codon;~~  
an orthogonal leucyl aminoacyl- tRNA synthetase (leucyl-O-RS), wherein the leucyl-O-RS preferentially aminoacylates the leucyl-O-tRNA with a selected amino acid; the selected amino acid; and,  
a nucleic acid that comprises a polynucleotide that encodes a polypeptide of interest, wherein the polynucleotide comprises at least one [[a]] selector codon that is recognized by the leucyl O-tRNA, and wherein the leucyl O-tRNA is derived from *Halobacterium sp NRC-1* and the leucyl O-RS is derived from *Methanobacterium thermautrophicum thermoaautotropicum*.

**27-61. (cancelled)**